Clusters of interacting receptors can stabilize synaptic efficacies

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Communicated by Leon N Cooper, Brown University, Providence, RI, August 15, 2005 (received for review April 6, 2005)

Synaptic weights store memories that can last a lifetime. Yet, memory depends on synaptic protein receptors that are recycled in and out of the membrane at a fast rate, possibly several times an hour. Several proposals to bridge this vast gap in time scales between memory and its molecular substrate have relied on bistable molecular switches. Here, we propose an alternative to this approach based on clusters of interacting receptors in the synaptic membrane. We show that such clusters can be metastable and that the lifetime of such clusters can be many orders of magnitude larger than the lifetime of the receptors of which they are composed. We also demonstrate how bidirectional synaptic plasticity can be implemented in this framework.

memory | model | synaptic plasticity

Synaptic efficacies depend on the number and conformational states of receptor proteins. However, receptors have a limited dwell time in the synaptic membrane, and they recycle in and out of the synapse possibly several times an hour (1, 2). Conformational changes due to phosphorylation are also short lived and can be reversed by phosphatases and receptor turnover. Yet, synaptic strengths are the basis of learning and memory processes that can persist a lifetime. The central difficulty in understanding the stability of memory and learning arises because synaptic efficacies must be uniquely regulated at the level of individual synapses. This observation rules out many possible mechanisms that are solely based on whole-cell processes, such as the regulation of gene expression.

The fundamental problem of preserving synapse-specific synaptic efficacies for long times, orders of magnitude larger than the lifetime of their molecular substrates, was pointed out by Francis Crick (3), who proposed a molecular switch as a likely solution. This idea was extensively expanded and investigated by John Lisman *et al.* (4, 5). Lisman hypothesized that this problem can be solved by a molecular switch in the signal transduction pathway that regulates synaptic efficacy and proposed a specific mechanism based on autophosphorylation of calmodulin-dependent PK II (CaMKII) holoenzymes. Modeling studies show that autophosphorylation of CaMKII results in a positive feedback loop that can keep the enzyme in an active state despite dephosphorylation by phosphatases and protein turnover (4, 5). The CaMKII hypothesis is appealing because it is well established that CaMKII and its autophosphorylation plays a key role in the induction of long-term potentiation (LTP) (6). However, there is no significant experimental evidence demonstrating that activation or autophosphorylation of CaMKII is necessary for the long-term maintenance of synaptic efficacies (7). Other components of the molecular signal transduction pathways controlling synaptic plasticity have also been proposed as possible molecular switches (8).

In this paper, I propose a theory in which the stability of synaptic efficacies is based on local interactions between receptors within a single synapse. Specifically, I propose that interactions between receptors within a cluster can alter the trafficking of receptors in and out of the synaptic membrane, thereby creating a metastable synaptic state that significantly increases the stability of synaptic efficacy without changing the mean dwell time of receptors in the synaptic membrane. The cluster theory proposed here is formally distinct from equilibrium theories of synaptic stability because it does not result in equilibrium states that are stable forever. The synaptic states generated by the cluster theory are metastable; at some point in time, these states will break and decay. However, the lifetimes of these metastable states are orders of magnitude larger than the lifetimes of their components.

This paper also demonstrates how bidirectional and synapse-specific long-term plasticity can be incorporated into the model. Finally, I show that statistical fluctuations in the number of receptors are a signature of this model that might be used to distinguish it from other synaptic models.

The cluster theory of synaptic stability is presented here in an abstract form. However, if the general principals of this theoretical model are found to be consistent with experimental evidence, identifying the molecular basis of the cluster model will become important.

Mathematical Methods

The variable S_{ij} is an occupation variable of the lattice site denoted by indices i and j. If the site is occupied, $S_{ij} = 1$; otherwise, $S_{ij} = 0$. Insertion of a new receptor into the membrane can occur at any unoccupied site in the lattice, and internalization of a receptor can occur only at occupied sites. In this formulation, internalization occurs at a fixed rate, independent of interaction with other receptors. I used a fixed internalization rate $\mu = 1/\tau_{\text{in}}$ per unit time, which implies that the probability of internalizing a receptor at site (i, j) in a small time step Δt is

$$P^{\text{in}}(i, j, t{:}t + \Delta_t) = S_{ij}\mu\Delta t. \quad [1]$$

Throughout this paper, we use $\mu = 1$, which implies that the mean dwell time of a receptor in the membrane is 1 unit of time. Typically, we use a time step $\Delta t < 0.01$, which is significantly smaller than the other time constants in this system.

Inserting a new receptor into an unoccupied site depends on the occupation in the vicinity of the unoccupied site. I calculate a "field" $h_k(i,j)$ at each unoccupied site i,j that measures the number of membrane-embedded receptors in the local neighborhood. The equation defining the field ($h_1(i,j)$) is described in Eq. **4**.

The field h_k will determine the conditional probability of inserting a new receptor into an unoccupied site. A typical parameter used in our simulations is $L_1 = 1.5$; however, similar stability is obtained for the range of $L_1 = 1.2$–2.0. The lattice repulsion constant used for the second population is $L_2 = 0.9$.

To determine insertion probability we use

$$P_k(i,j) = 1/(1 + \exp(-\beta h_k(i, j))), \quad [2]$$

which varies smoothly from 0 to 1 as a function of $h_k(i, j)$. The constant β is the slope of this function. Stability increases for larger β. I typically use $\beta = 50$. However, values of $\beta > 25$ are sufficient for stability of up to ≈1,000 time steps, with 49 receptors in the

Abbreviations: LTP, long-term potentiation; LTD, long-term depression; CaMKII, calmodulin-dependent PK II; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

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www.pnas.org/cgi/doi/10.1073/pnas.0506934102

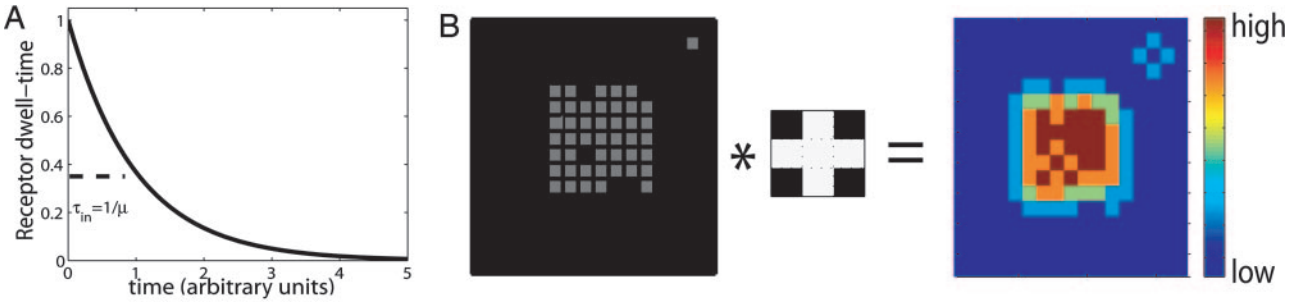

Fig. 1. Model assumptions. (*A*) Receptors in the synapse are internalized stochastically at a constant rate, and their probability of staying in the synapse decays exponentially with a time constant of 1. (*B*) The rate of insertion depends of the number of nearest neighbors. Given the occupation state (*Left*), a field is calculated (*Right*). The probability of inserting a new receptor is proportional to this field. The field can be computed from convolving the nearest-neighbor function (*Center*) with the state. The field is higher within the cluster and close to its boundaries than outside the cluster or near the isolated receptor.

initial state. This stability depends on other parameters, such as L_1. The probability of inserting a receptor in an unoccupied site in a very small time step Δt is then

$$P_k^{\text{ex}}(i,j) = (1 - S_{ij})(\rho_k r \Delta t P_k(i,j)), \qquad [3]$$

where ρ_k is the probability that a receptor of type k is present in a position near the empty site, and r is the rate of transition into the empty site. Typically, we use $\rho_1 = 0.95$ and $r = 10$, which implies that for $P_k \approx 1$, the average time for inserting a receptor into a vacant site with a high h_k is ≈0.1 units of time, significantly faster than the internalization rate and slower than the typical time step used. Eq. **3** is arrived at for small Δt by approximating the expression for finite Δt: $\rho_k r \Delta t P_k(i,j) \approx 1 - \exp(\rho_k r \Delta t P_k(i,j))$.

The key to stability is not the identity of specific parameters, such as ρ_k and r, but their consequence that the characteristic time for insertion into an empty site in a cluster is much shorter than the characteristic time of removing a receptor from a cluster.

To reduce run time, we use parallel dynamics. The use of parallel dynamics is not a problem because we use small time steps in which a very small number of events occur across the whole lattice. I ran a few random sequential simulations and obtained indistinguishable results.

Results

The cluster theory of synaptic stability is based on several assumptions: (*i*) Synaptic efficacy is proportional to the number of postsynaptic receptors [for example, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors]. (*ii*) Receptors in the postsynaptic density are clustered. (*iii*) The insertion rate of a receptor in the vicinity of other receptors in the cluster is much higher than for an isolated receptor. (*iv*) The rate of receptor removal from the cluster is independent of interactions with other receptors in the cluster. Assumptions *i–iii* are essential assumptions of this model, whereas assumption *iv* could be altered while preserving the main features of the model. It is important to note that the insertion rate (on rate) and removal rate (off rate) are controlled independently and not governed by a single parameter, which is important for the robust functioning of the model and makes it formally distinct from an Ising spin model of statistical physics (9) (see section 3 of *Supporting Text*, which is published as supporting information on the PNAS web site).

The effect of assumption *iv* is that the dwell time of a receptor in a cluster is the same as that of an isolated receptor and is independent of cluster interactions. From a biophysical perspective, it might seem more plausible that the on rate is constant and the off rate is neighbor-dependent. Section 2 of *Supporting Text* examines the consequences of this off-rate model and shows that it extends the lifetime of clusters by extending the lifetime of the single receptors, thus not really addressing the problem. I discuss below how the on-rate model presented here might be justified on a biophysical basis.

For simplicity of implementation, the model is implemented on a square grid. Insertion and removal of synaptic receptors is based on the following specific sequence of events. First, at each time step (Δt) and for each synaptic site occupied by a receptor, the receptor can be randomly removed from the cluster with a probability $\mu \Delta t$. This random removal implies that each receptor has a mean dwell time of $\tau_{\text{in}} = 1/\mu$ and that its average kinetics are exponential (Fig. 1*A*); I typically use $\mu = 1$, so that times here are measured in units of the mean dwell time of synaptic receptors. Next, at each unoccupied receptor site, a field h_1 is calculated such that its value is the number of occupied neighboring sites minus a lattice repulsion constant L_1.

$$h_1(i,j) = \left(\sum_{lm} S_{l,m} I(l - i, m - j) - L_1 \right), \qquad [4]$$

where i, j and l, m are indices of sites in a two-dimensional synaptic surface and $S_{i,j}$ is an occupation variable of the site labeled by indices i and j that is 1 if a site is occupied and 0 if not. The function I is an interaction function. A simple example of an interaction function is the nearest-neighbor function, which assumes that only the four nearest neighbors contribute and has the form

$$I(i,j) = \begin{cases} 1 & \text{if } i^2 + j^2 = 1 \\ 0 & \text{if } i^2 + j^2 \neq 1. \end{cases} \qquad [5]$$

Throughout this paper, I assume this simple nearest-neighbor interaction function, which could easily be generalized to more complex local functions.

An example of the field calculated for a specific occupation pattern is given in Fig. 1*B*. The field (h_1) calculated determines the probability of inserting a new receptor into an empty site. I implement assumption *iii* by setting the probability of insertion to be a steeply increasing monotonic function of this field (see Eq. **2**). The primary effect of this implementation is that the insertion probability at a site with many neighbors (within a cluster or on its boundary) is orders of magnitude higher than for a site with a small number of neighbors.

Stability characteristics are demonstrated by simulations of cluster dynamics (Fig. 2). Starting from an initial state of 49 receptors (a square of 7×7 receptors), single receptors are randomly internalized, and new ones are inserted in their place, resulting in a fluctuating number of receptors at each time step. Examples of the receptor configurations at different time steps are shown in Fig. 2*A* (see Movie 1, which is published as supporting information on the PNAS web site). The number of receptors in such a cluster fluctuates around stable mean, which is preserved for periods of

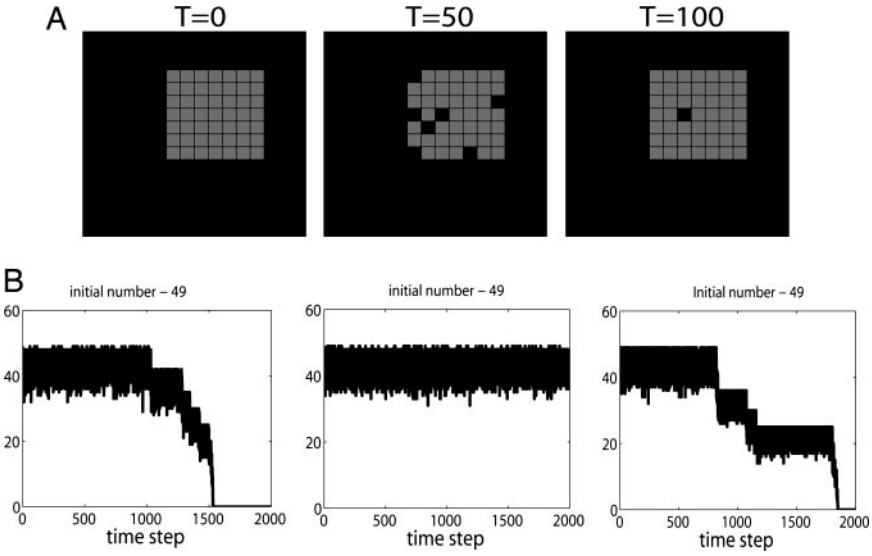

Fig. 2. Metastable clusters of receptors. (*A*) States of a cluster of receptors at different times from a single simulation. (*Left*) The initial state ($T = 0$). (*Center* and *Right*) Two additional times, $T = 50$ and $T = 100$. The initial state was a square with dimensions of 7×7 receptors. Gray squares represent a site occupied with a receptor; black represents an unoccupied site (see Movie 1). (*B*) The number of receptors as a function of time for three different simulations with the same initial conditions. The number of receptors in a cluster continuously fluctuates. Occasionally, the cluster size exhibits a jump downward, which occurs by a deletion of a complete edge.

time that are orders of magnitude longer than the lifetime of any single receptor in the cluster. Different simulations with a different sequence of random events produce different cluster dynamics and lifetimes (Fig. 2*B*).

All simulations in this paper were carried out with a square grid. However, different grid structures can also support metastable states. For example, see Movie 2, which is published as supporting information on the PNAS web site, in which stability in a triangular grid is demonstrated.

The metastability of the clusters is parameter-dependent. In section 1 of *Supporting Text* and in Fig. 7, which are published as supporting information on the PNAS web site, I show the parameter region (β, L_1) that supports metastability. For example, at $\beta = 50$, a lattice repulsion constant that is too large ($L_1 > 2.0$) causes the clusters to rapidly shrink, and if it is too small, ($L_1 < 1.2$) clusters will grow. In general, the metastable region exists for values of intermediate values of L_1 and is wider for higher β. Deterministic insertion ($\beta \to \infty$) would maximize the metastable region and the lifetime of clusters in this region. For stability, it is important that the interaction between receptors are short range. Apart from nearest-neighbor interactions, other short-range interaction functions, which fall rapidly enough with distance, support metastability as well.

Cluster instability originates at the boundaries where there are fewer nearest neighbors. Change in the synaptic state usually results from the deletion of an entire edge of a cluster. Therefore, jumps in cluster size typically have magnitudes of $\approx\sqrt{N}$, where N is the number of receptors in the cluster. Because the ratio between the area of a cluster and its perimeter falls with cluster size, cluster stability is likely to be size-dependent. Kinetics of clusters with a different number of receptors in the initial condition (Fig. 3 *A–C*) demonstrate the size dependence of cluster lifetime and the discrete jumps in cluster size. The size dependence of cluster lifetime is summarized in Fig. 3*D*, where lifetime is defined as the time before the first downward shift to a new metastable state.

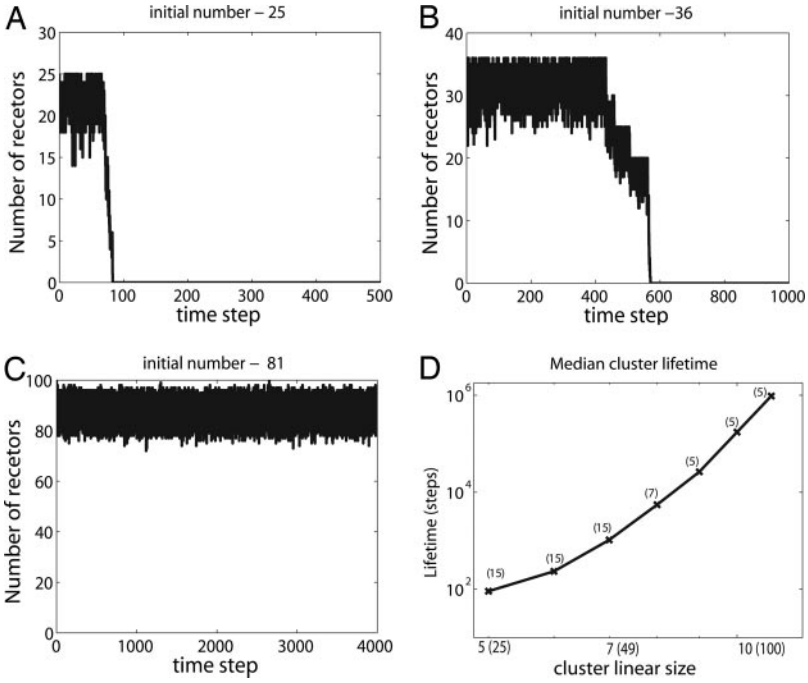

Fig. 3. Cluster lifetimes depend on their initial size. (*A–C*) Shown are simulations displaying receptor number vs. time in three different simulations with three different initial sizes: 25 (*A*), 36 (*B*), and 81 (*C*). (*D*) A summary of median cluster lifetimes as a function of size. The *x* axis gives the linear size, which is the square root of the initial number of receptors. Both the *x* and *y* axes are in logarithmic scale. Cluster lifetime is defined as the time until the first downward jump. Results are medians over a number of independent simulations (numbers indicated above each data point).

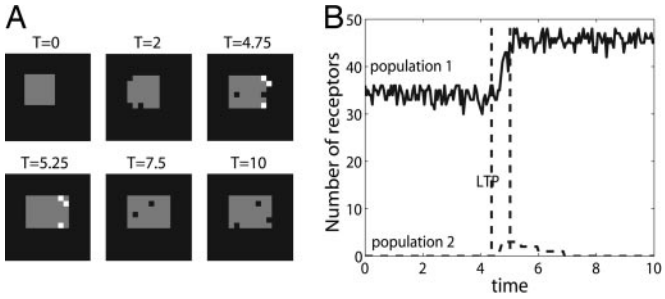

Fig. 4. Induction of LTP using the cluster model, based on the activation of an additional population of receptors with a lower repulsion constant. (*A*) The temporal evolution of a cluster. Gray squares represent the first population of receptors, and white represents the second population. The second population is active from 4.5 to 5 time steps. This brief activation of the second population causes an increase in the cluster size and is rapidly replaced with the first population once inactivated (see Movie 3). (*B*) The total number of receptors in the cluster (solid line) and receptors of the second population (dashed gray line) for the same simulation as in *A*. The vertical dashed lines indicate the time period in which the second population is active.

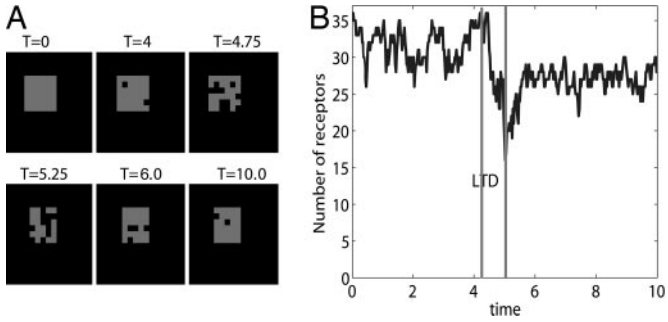

Fig. 5. Induction of LTD by a transient increase of receptor internalization. (*A*) Temporal dynamics of a cluster in which the receptor internalization rate is increased by a factor of 4 between $T = 4$ and $T = 5$ (see Movie 5). (*B*) The total number of receptors in the cluster as a function of time during the induction of LTD.

The median receptor lifetime of a 9×9 cluster is ≈25,000 times the dwell time of a single receptor. The actual dwell time of receptors in a real synapse is not well known. Some indirect methods produced surprisingly short estimates of 20 min (1, 2). Assuming a receptor dwell time of 20 min and a cluster with an initial size of 9×9 receptors produces an estimate that the median cluster lifetime is >1 year.

Bidirectional Synaptic Plasticity. Synaptic efficacies should be stable, but it must also be possible to bidirectionally alter them in a synapse-specific manner. Both LTP and long-term depression (LTD) are synapse-specific and stable over long periods of time (10, 11), and such synapse specificity is necessary for the ability to store memories and learning.

LTP can be implemented in the cluster model in various ways. One possibility is to introduce the hypothesis that the induction of LTP activates a second population of receptors with a higher probability of insertion on the external boundaries of a cluster. Mathematically, this form of LTP is implemented by assuming a smaller repulsion constant for this population. Assuming $L_2 = 0.9$ for this population, a single neighbor is sufficient for insertion, and zero neighbors are insufficient. I assume that this second population is only very transiently activated, and at a low concentration.

Simulation results displaying the induction of LTP are shown in Fig. 4 (and Movie 3, which is published as supporting information on the PNAS web site). The second population of receptors, displayed in white, is transiently activated between $T = 4.5$ and $T = 5$. Receptors from the second population are added primarily on the boundaries of the original cluster ($T = 4.75$), resulting in growth of the original cluster by addition of new lines or rows ($T = 5.25$). After the second population is inactivated, it is rapidly replaced by receptors of the first constitutive population ($T = 7.5$). The solid line in Fig. 4*B* shows the total number of receptors as a function of time, and the dashed line shows the number of receptors from the second, transient population.

LTP can also be induced with only one population of receptors by transiently reducing the value of L_1. In Movie 4, which is published as supporting information on the PNAS web site, we show an example in which LTP induction is accomplished by reducing L_1 to 1.0 between the times $T = 4.95$ and $T = 5.0$.

One consequence of using these protocols for LTP is that the probability of inducing LTP with a transient stimulus increases with the size of the current cluster. However, the relative minimal size of an upward jump is smaller. These consequences might be altered if additional assumptions are made about induction. For example, the number of intracellular receptors available at a synapse might be smaller for larger synapses, thus reducing the probability of LTP in large synapses. The computational consequences of the size dependence of the probability of inducing LTP and the relative magnitude of an LTP event are yet to be investigated.

Reduction of synaptic efficacies, LTD, has been implemented in several ways. A simple implementation is to transiently increase the endocytosis rate, which is implemented here by setting the endocytosis time constant to $\tau = 1/4$. Fig. 5 (and Movie 5, which is published as supporting information on the PNAS web site) shows simulation results of the induction of LTD using this method. Typically, this method results in a large transient decrease in synaptic efficacy followed by a partial recovery. We have examined another method of inducing LTD that depends on transiently increasing L_1. This method produces qualitatively similar results but differs in details such as the magnitude of the transient decrease and the typical induction time of LTD (Movie 6, which is published as supporting information on the PNAS web site).

Fluctuations of Metastable States and Comparison to an Equilibrium Model. The ultimate experimental test for the cluster model would be to directly examine its hypotheses, such as the assumption that insertion of new receptors near other receptors is much more likely than insertion in an isolated site. Direct confirmation of this hypothesis requires imaging at a resolution beyond the available technological limits.

Another approach is to test consequences of the model that differentiate it from alternative models. I have chosen to examine fluctuations from the mean during a metastable state in our model and compare them to fluctuations of an equilibrium model. The advantage of using an equilibrium model for comparison is that its predicted fluctuations can be calculated analytically (section 4 of *Supporting Text*).

I assume a simple equilibrium model for receptor trafficking in and out of the synapse. In this simple model, the total population of receptors is composed of two subpopulations, a synaptic population (R_s) and a nonsynaptic population (R_i), such that the total number of receptors $R_s + R_i = R_T$ is fixed. The kinetic diagram describing this process is

$$R_i \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} R_s, \qquad [6]$$

where K_1 and K_{-1} are the forward and backward kinetic coefficients. Although the kinetic coefficients and the total number of receptors R_T might change as a function of synaptic plasticity, here I analyze the dynamics and fluctuations of the equilibrium model assuming that the kinetic coefficients and R_T remain constant.

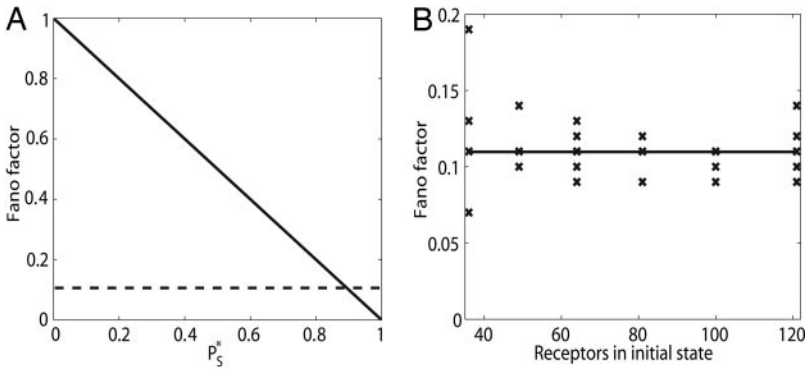

Fig. 6. The Fano factor for the equilibrium and cluster models. (*A*) The Fano factor for the equilibrium model plotted from Eq. **4**. As the fraction of receptors in the synaptic state (P_S^*) increases from 0 to 1, the Fano factor decreases from 1 to 0. (*B*) Fano factor for the cluster models calculated from the metastable state of simulations with various initial sizes from 6×6 to 11×11. The Fano factor shows no significant dependence on size and has a mean of 0.11 (solid line) over all simulations carried out. Five simulations were performed for each initial state, but some of the data points overlap.

The equilibrium model does not address the central issue examined in this paper: how to obtain stable, synapse-specific synaptic efficacies. In the equilibrium model, bidirectional synaptic plasticity can be obtained by changing the kinetic coefficients or the total number of receptors. However, it is not clear how these variables could be changed by a brief and transient plasticity paradigm but maintained for prolonged periods of time. Presumably, this prolonged change would be controlled by molecular processes upstream from the receptors, which are not specified here.

I use the Fano factor, defined as the variance/mean of the receptor number, as a statistical measure of fluctuations. For the equilibrium model,

$$F = (1 - P_S^*), \qquad [7]$$

where P_S^* is the probability of a receptor being in a synaptic state, or, equivalently, the fraction of receptors in the synaptic pool (see section 4 of *Supporting Text*). The dependence of F on P_S^* is shown in Fig. 6*A*.

The Fano factor for the cluster model was calculated for multiple simulations with different initial sizes (Fig. 6*B*). I carried out five simulations for each initial size and calculated the Fano factor over the initial metastable state. The Fano factor in the cluster model seems independent of the initial size, with an average value over all conditions of 0.11. If the Fano factor were calculated over periods of time spanning transitions from one metastable state to another, it would have a significantly higher value. For the equilibrium model to have Fano factors as small as those of the cluster model, we would have to assume that ≈90% of the receptors reside in the synaptic pool. This prediction is measurable and could be used to distinguish between these two different models. However, the technique used to assess the number of synaptic receptors and their fluctuations must have very small measurement errors, as not to obscure the variability of the receptor number.

Discussion

The cluster model presented in this paper is proposed as a possible mechanism for long-term stability of synaptic efficacies. I have demonstrated that a synapse formed from a cluster of interacting receptors can have stable efficacies for periods of time that are several orders of magnitude larger than the dwell time of any single receptor in the cluster and that the lifetime of clusters increases rapidly with the number of receptors in the cluster. Estimates of the number of AMPA receptors in a synapse using anatomical methods are on the order of 50–100 (12). Physiological methods estimate that the number of postsynaptic AMPA receptors on spines of CA1 neurons are on the order of 60–190 (13, 14). A cluster with an initial state composed of 81 receptors, well within the plausible range, has a median lifetime of >25,000 time steps. Experiments in which receptor dwell time is indirectly monitored by using overexpression of tagged GluR2 receptors in a slice result in estimated receptor dwell times of 10–30 min (1, 2). Using a receptor dwell time of 20 min, we find that a cluster with 81 receptors in the initial state has a lifetime of >1 year. Although a cluster lifetime of 1 year is still significantly less than the lifetime of memories in a human, the cluster model could be a mechanism to bridge a significant portion of the gap between receptor dwell times and the lifetime of memory. However, because the estimates of receptor dwell time are indirect and because the system used has several properties that could alter the result with respect to a synapse *in vivo*, it might be possible that the real receptor dwell time is much larger. If the receptor dwell time were 1 day, the cluster lifetime would be >65 years.

The model I have presented here is very abstract, and I do not attempt to provide a molecular mechanism that could account for the insertion and removal of receptors. There are many possible mechanistic implementations that could fall under the same family of models. The entity we call a receptor might be a single receptor, but it may also be the complex of a receptor and its associated proteins, or it might include more than one receptor. The removal and insertion of a receptor might be carried out by endocytosis and exocytosis (15) but also by diffusion of receptors within the synaptic membrane (16, 17). The interactions between receptors might be mediated by direct forces between membrane-embedded receptors, the same type of interactions that might lead to aggregation of different proteins (18). However, these interactions are quite likely to depend on the more complex network of postsynaptic proteins associated with the receptors (19, 20), in which case, the properties of these dynamics will be largely independent of the underlying physics of protein aggregation.

In the cluster model proposed here, the receptor's on rate is neighbor-dependent, and the off rate is constant. If the clusters are viewed as aggregates, it might seem that it is more natural to assume the opposite. I have examined the consequences of this off-rate model, and in section 2 of *Supporting Text*, I demonstrate that although it does extend the lifetime of clusters, it does so by extending the dwell time of receptors, not by extending the ratio of cluster lifetime to receptor dwell time. What are possible mechanisms that could support an on-rate model? A constant off rate could result if the molecular machinery that internalizes receptors operates primarily on "tagged" receptors, where the tag might correspond to their phosphorylation state (21). If this tagging procedure proceeds at a fixed rate, then the off rate will be constant. A neighbor-dependent on rate could come about if the nearby receptors act to somehow reduce the energetic cost of inserting a receptor in their vicinity, which could occur directly by electromagnetic shielding or indirectly through the network of synaptic proteins linked to the receptors. Another possibility is that the on rate is an effective rate brought about by the formation of a diffusive trap caused by clusters. This alternative is consistent with recent experimental findings that show that receptors diffuse at two distinct rates, with a much lower rate spatially coincident with locations of synaptic contacts (16, 17). The consequences of this diffusive trap

model are not trivial, and homology with the cluster model is not evident.

Different mechanisms of interaction between receptors, and receptor trafficking, could imply significantly different specific properties and lifetimes for receptor clusters. For example, if insertion and removal are carried out by exocytosis and endocytosis of groups of several receptors, then the effective number of "receptor units" in a cluster would be lower, and the lifetime of clusters might be significantly lower. If receptors in the cluster are exchanged by diffusion through the membrane, exchange of receptors would occur only through the cluster boundaries, which might alter the stability characteristics of receptor clusters. Although different mechanisms might have significantly different quantitative consequences, the central aim of this paper is not to identify the exact biophysical mechanisms but to propose a different idea at a more abstract level.

The cluster model is an alternative to theories of synaptic stability that rely on a molecular switch (3, 4, 5). It differs fundamentally from molecular switch theories because it does not depend on the existence of bistable states of chemical equilibrium. It also proposes an alternative to more recent theories that depend on prion-like properties of synaptic proteins (22). The cluster theory depends on interactions within clusters of receptors and is therefore fundamentally different from theories that are based on the state of single proteins, independent of their interactions. The theory is general enough to allow that different types of mechanisms might exist in the same synapses, possibly to account for stability on different time scales.

Previously, a receptor mosaic hypothesis proposed that receptor aggregates in the postsynaptic and presynaptic membrane can form a computational molecular network (23, 24). Although the assumptions of this hypothesis are reminiscent of the cluster model, it does not address the issue of synaptic stability, the central challenge addressed by the cluster model.

Although I am not offering a detailed mechanistic description of the cluster model, it is possible to identify potential molecular building blocks of the model and of the bidirectional plasticity of receptor clusters. One possible interpretation is that the first stable population of AMPA receptors is composed of GluR2/3 heteromers. This interpretation is consistent with the observation that AMPA receptors composed of GluR2 subunits are constitutively recycled in and out of the synapse in an activity-independent manner (15, 2). According to this interpretation, the second population of receptors, necessary for LTP, are heteromers that include a GluR1 subunit that are delivered to synapses in an activity-dependent manner (2). If LTD is implemented by increasing the rate of endocytosis of AMPA receptors, this process could be facilitated by phosphorylation of the serine 880 site on the GluR2 subunit, which increases the internalization rate of these receptors (21). Another possible interpretation is that the receptors are heteromers that include the GluR1 receptor and that these receptors are constitutively phosphorylated at serine 845 (25–28). Induction of LTP is achieved by transient phosphorylation of GluR1 receptors at both serine 845 and serine 831, which reduces the value of their lattice repulsion constant (L_1). This doubly phosphorylated conformational state is known to be associated with the induction of LTP (27). According to this interpretation, the induction of LTD is obtained by dephosphorylation of both serine 831 and 845 sites (26, 29), a condition associated with an increased internalization rate of AMPA receptors. These two different interpretations are not mutually exclusive and can possibly complement each other.

Experimental tests of the fundamental postulate of this model, that insertion of a new receptor is more likely in the vicinity of other receptors, are difficult because the diffraction limit makes it difficult to resolve single receptors optically. I have proposed an alternative test that relies on fluctuation analysis of receptor numbers. This test is based on the finding that the cluster model has relatively small fluctuations compared with an equilibrium model. Experimental techniques to examine this fluctuation must have a low variability in their measurements to avoid a significant overestimate of the variability in the sample. It is currently hard to find physiological or anatomical data that have sufficient precision to test receptor number fluctuations. I am hopeful that publication of this theory will encourage experimental groups to devise tests for the assumptions or consequences of this model.

I thank members of the Institute for Brain and Neural Systems at Brown University (Providence, RI), Jeff Gavornik and Yidao Cai (both of University of Texas), and the two reviewers for very helpful comments on the manuscript.

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